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APPLICATION FOR UNITED STATES LETTERS PATENT

FOR

TRANSGENIC PLANTS EXPRESSING LEPIDOPTERAN-ACTIVE δ-ENDOTOXINS

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1.0 BACKGROUND OF THE INVENTION

The present invention is a continuation-in-part of United States Patent Application Serial Number 08/757,536, filed November 27, 1996, the entire contents of which is specifically incorporated herein by reference.

1.1 FIELD OF THE INVENTION

The present invention relates generally to the fields of insect control. Certain embodiments concern methods and compositions comprising nucleic acid segments which encode *Bacillus thuringiensis*-derived δ-endotoxins. Disclosed are methods of altering Cry1 crystal proteins by mutagenesis of the loop regions between the α-helices of the protein's domain 1 or of the loop region between α-helix 7 of domain 1 and β-strand 1 of domain 2 to give rise to modified Cry1 proteins (Cry1*) which have improved activity against Lepidopteran insects. Various methods for making and using these recombinantly-engineered proteins and nucleic acid segments, including development of transgenic plant cells and recombinant host cells are also disclosed.

1.2 DESCRIPTION OF THE RELATED ART

The most widely used microbial pesticides are derived from the bacterium *Bacillus thuringiensis*. *B. thuringiensis* is a Gram-positive bacterium that produces crystal proteins which are specifically toxic to certain orders and species of insects. Many different strains of *B. thuringiensis* have been shown to produce insecticidal crystal proteins. Compositions including *B. thuringiensis* strains which produce insecticidal proteins have been commercially-available and used as environmentally-acceptable insecticides because they are quite toxic to the specific target insect, but are harmless to plants and other non-targeted organisms.

δ-endotoxins are used to control a wide range of leaf-eating caterpillars and beetles, as well as mosquitoes. *B. thuringiensis* produces a proteinaceous parasporal body or crystal which is toxic upon ingestion by a susceptible insect host. For example, *B. thuringiensis* subsp. *kurstaki* HD-1 produces a crystal inclusion comprising δ-

endotoxins which are toxic to the larvae of a number of insects in the order Lepidoptera (Schnepf and Whiteley, 1981).

1.2.1 δ-ENDOTOXINS

δ-endotoxins are a large collection of insecticidal proteins produced by *B. thuringiensis*. Over the past decade research on the structure and function of *B. thuringiensis* toxins has covered all of the major toxin categories, and while these toxins differ in specific structure and function, general similarities in the structure and function are assumed. Based on the accumulated knowledge of *B. thuringiensis* toxins, a generalized mode of action for *B. thuringiensis* toxins has been created and includes: ingestion by the insect, solubilization in the insect midgut (a combination stomach and small intestine), resistance to digestive enzymes sometimes with partial digestion actually "activating" the toxin, binding to the midgut cells, formation of a pore in the insect cells and the disruption of cellular homeostasis (English and Slatin, 1992).

1.2.2 GENES ENCODING CRYSTAL PROTEINS

Many of the δ-endotoxins are related to various degrees by similarities in their amino acid sequences. Historically, the proteins and the genes which encode them were classified based largely upon their spectrum of insecticidal activity. The review by Höfte and Whiteley (1989) discusses the genes and proteins that were identified in *B. thuringiensis* prior to 1990, and sets forth the nomenclature and classification scheme which has traditionally been applied to *B. thuringiensis* genes and proteins. *cryI* genes encode lepidopteran-toxic CryI proteins. *cryII* genes encode CryII proteins that are toxic to both lepidopterans and dipterans. *cryIII* genes encode coleopteran-toxic CryIII proteins, while *cryIV* genes encode dipteran-toxic CryIV proteins.

Based on the degree of sequence similarity, the proteins were further classified into subfamilies; more highly related proteins within each family were assigned divisional letters such as CryIA, CryIB, CryIC, *etc.* Even more closely related proteins within each division were given names such as CryIC1, CryIC2, *etc.*